Abstracts

Gene regulatory network underlying neural crest formation

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The neural crest is a population of multipotent, migratory progenitor cells that forms at the border of neural and non-neural ectoderm in vertebrate embryos. These cells then migrate from the neural tube along defined pathways, populate numerous sites and differentiate into diverse cells types including melanocytes, sensory and autonomic neurons, and mineralized matrices like bone and dentine. Data compiled from Xenopus, zebrafish, mouse and chick, suggest that a network of interacting transcriptional regulators and downstream effector genes confer properties like multipotency and migratory capacity to nascent neural crest cells. These regulatory interactions can be divided into distinct phases. The first involves inductive signals (e.g. Wnt, BMP, FGF) that establish the neural plate border, by up-regulation of border specifier genes like Msx1/2, Pax3/7, and Zic. These border genes in turn up-regulate neural crest specifier genes like Slug/Snail, FoxD3 and the SoxE family. Finally, the neural crest specifiers turn on specific downstream targets that render the neural crest migratory and multipotent. We are testing linkages in this hypothetical neural crest gene regulatory network in chick and lamprey by systematically perturbing a subset of the transcription factors involved in early neural crest specification and examining the effect of these perturbations on likely downstream genes in order to test the predicted interrelationships. In addition, we are isolating cis-regulatory regions of genes in this putative neural crest regulatory network to identify neural crest enhancers, determine additional inputs to the network and determine which interactions are direct. The results suggest that a series of gene regulatory circuits are involved in the production of migratory neural crest cells in the early vertebrate embryos and that many of these events may be conserved to the base of vertebrates.

Characterization of the Stathmin SCG10 and its interactions with Kinesin Binding Protein (KBP) in zebrafish

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SCG10 (STMN2) is believed to be neuronal-specific stathmin that is enriched in the growth cones of developing neurons and has a role in neurite outgrowth. In all species so far examined SCG10 is expressed in both the CNS and PNS. Recently we have shown that SCG10 interacts with the KBP (KIAA1279) protein. Mutations in kbp have been shown to be responsible for human Goldberg-Shprintzen (GOSHS) syndrome. Patients with this rare autosomal recessive disorder manifest microcephaly, mental retardation, polymicrogyria, facial dysmorphisms and Hirschsprung disease. The precise function of KBP in nervous system development is currently not known. To begin to understand the function of SCG10 we have determined the temporal and spatial expression pattern of the two zebrafish SCG10 orthologues, SCG10a and SCG10b, by RT-PCR and in situ hybridization. RT-PCR shows that both transcripts are maternally deposited and are detectable from 0 hpf through 5 days – the latest age we examined. *In situ* hybridization analysis reveals that the pattern of expression of these two genes is dynamic and spatially restricted but is nearly identical for both homologues. SCG10a and b are principally restricted to the CNS from 24–48 hpf. From 48 hpf onwards expression of both genes becomes restricted to the anterior CNS and cranial ganglia. This pattern of SCG10 expression at 48 hpf resembles the pattern of KBP gene expression, which is limited to the anterior CNS from 48 hpf onwards. Subsequently, both SCG10 genes are expressed additionally in the developing gut from 72 hpf. To investigate the *in vivo* function of the zebrafish SCG10 orthologues and their interactions with KBP, we generated morpholinos (MOs) for all three genes. Morphant embryos have a severe phenotype at relatively low MO concentration. It is likely that there is redundancy between the two SCG10 genes as double SCG10 morphants have a more severe phenotype when compared to single morphants. Significantly, triple morphants injected with sub-threshold doses of SCG10 and KBP MOs display a severe phenotype, strongly suggesting an epistatic interaction between the products of these genes. Our data suggests that SCG10 and KBP genes are required for proper differentiation of pharyngeal arches, migration of ENS precursors and later differentiation stages of the cranial ganglia.

Function of the RNA binding protein HERMES in gastrointestinal morphology and motility

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The motility of the digestive tract is ensured by the correct coordination of the autonomous enteric nervous system (ENS) and the visceral smooth muscle cells (SMC). The ENS originates from neural crest cells that migrate from the dorsal region of the neural tube and colonize the whole gut to establish its innervation. The SMCs derive from the splanchnopleural mesoderm that will form the undifferentiated visceral mesenchyme before their final differentiation. Motility disorders in infants comprise a wide group of heterogeneous diseases. Hirschsprung disease (HSCR) is a particular case due to an absence of the ENS along certain lengths of the bowel. The SMC differentiation is also often affected in patients with congenital gut malformations and motility disorders such as Chronic Intestinal Pseudoobstruction (CIP). However, few have investigated the status of SMC in physiopathological conditions. Our aim was to investigate the molecular mechanisms that control the differentiation of the visceral mesenchyme into SMCs in vertebrates. In order to identify these candidate pathways, we developed and analyzed the gene expression profiles of undifferentiated and differentiated avian stomach by microarray. We identified one new candidate HERMES, a RNA binding protein and examined the function of this factor during the development and the differentiation of the SMC structure by performing exhaustive *in vivo* and in primary SMC/ENS culture positive and negative approaches. We found that HERMES control different aspects of the SMC differentiation and deregulation of its expression and function alter the development of both SMC and ENS systems. In addition, we analyzed the expression of homologous HERMES gene in human physiopathological conditions. All these data demonstrate the necessity of correct coordination between the development/differentiation of the SMC and ENS and the importance to focus on both motility effectors.

Development of the mucosal plexus of the human enteric nervous system

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Introduction: Knowledge regarding the structure and development of the enteric nervous system (ENS) is crucial for the understanding of gut function and its disorders. It is widely accepted that the ENS is composed of two major plexus (myenteric and submucosal plexus) arranged as concentric rings in the bowel wall. There is, however, accumulating evidence from both animal studies, and our recent work in humans, that nerve cells also reside within the post-natal intestinal mucosa (mucosal plexus). The aim of this study was to examine the development-dependent formation of an intrinsic mucosal plexus of